

A prompt and favorable examination on the merits is earnestly solicited. The Examiner is invited to contact the undersigned representative to discuss any matter with respect to this application.

Respectfully submitted,



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Attachments:

Appendix  
Abstract

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## APPENDIX

## Changes to Abstract:

The following is a marked-up version of the amended Abstract.

~~Method for In-vitro Testing of Active Substances, Device and Its Use~~

## Summary:

ABSTRACT

A method for in-vitro testing of active substances on cells ~~comprises at least~~ includes the first steps ~~for~~ of providing a cell culture container with an interior chamber, ~~and~~ an inside wall, and ~~with~~ a first and second membrane system located in the interior chamber, ~~with a~~ A cell culture chamber is formed between the membrane systems and the inside wall of the interior ~~space~~ chamber. The method includes providing a cell culture and cell culture medium in a cell culture chamber, supplying a fluid nutrient medium to the cell culture chamber, and carrying away metabolic products by means of the first membrane system, ~~adding at~~ At least one gaseous medium is added to the cell culture chamber by means of the second membrane system, ~~adding and~~ at least one active substance is added to the cell culture chamber, ~~with the supplying taking~~ The supplying takes place according to an adjusted active substance concentration-time curve and monitoring cell vitality. In addition, a device and the use of said device for testing the effect of idarubicin on the leukemic cell line CCRF CEM is described.

## Changes to Specification:

The following is a marked-up version of the amended paragraphs:

[0001] ~~Description:~~

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] The invention relates to a method for testing active substances on cells in-vitro, a device, and ~~its use~~ a process for testing employing the device.

## Changes to Claims:

The following is a marked-up version of the amended claims:

1. (Amended) ~~Use of a~~ A method for in-vitro testing of active substances in cells comprising at least the following steps:
  - a) ~~Providing~~ providing a cell culture container with an interior ~~space~~ chamber and an inside wall and with a first and second membrane system located in the interior ~~space~~ chamber, whereby a cell culture space is formed between the first and second membrane systems and the inside wall of the interior chamber;
  - b) ~~Providing~~ providing cells as a cell culture and a cell culture medium in the cell culture ~~chamber~~ space;
  - c) ~~Adding~~ adding a fluid nutrient medium to the cell culture ~~chamber~~ space and removing metabolic products from the cell culture ~~chamber~~ space by means of the first membrane system;
  - d) ~~Adding~~ adding at least one gaseous medium to the cell culture ~~chamber~~ space by means of a the second membrane system;
  - e) ~~Metering~~ metering at least one active substance into the cell culture ~~chamber~~ space, with the metering taking place according to an adjusted active substance concentration-time curve; and
  - f) ~~Monitoring~~ monitoring cell vitality.

~~for in-vitro testing of active substances in cells.~~

2. (Amended) Use The method according to ~~Claim~~ claim 1, characterized in that wherein the active substances comprise cytostatics, antibiotics, cytokines, growth factors, or antiviral agents ~~are used as active substances~~.

3. (Amended) Use The method according to ~~one or more of Claims~~ claim 1 or 2, characterized in that wherein the cell culture comprises primary cells ~~are added as the cell culture~~.

4. (Amended) Use The method according to ~~one or more of Claims~~ claim 1 or 2, characterized in that wherein the cell culture comprises tumor cell lines ~~are used as the cell culture~~.

5. (Amended) Use The method according to ~~one or more of Claims~~ claim 1 to 4, characterized in that wherein the cell culture chamber space has a minimum volume of at least 0.1 ml ~~minimum~~ and a maximum volume of 5 ml ~~maximum~~.

6. (Amended) Use The method according to ~~Claim~~ claim 5, characterized in that wherein the cell culture chamber space has a minimum volume of 0.3 ml and a maximum volume of 3.0 ml.

7. (Amended) Use The method according to ~~one or more of Claims~~ claim 1 to 6, characterized in that wherein the first membrane system comprises at least one semipermeable membrane or at least one hydrophilic microporous membrane ~~is used as the first membrane system~~, and the second membrane system comprises at least one gas transfer membrane ~~is used as the second membrane system~~.

8. (Amended) Use The method according to ~~one or more of Claims~~ claim 1 to 7, characterized in that wherein the first and the second membrane systems consist of comprise hollow fibers stacked in multiple layers.

9. (Amended) Use The method according to ~~one or more of Claims claim 1 to 8, characterized in that a~~ wherein the cell culture container is used which has comprises a removable lid and allows the cell culture ~~to be prepared~~ is provided by adjusting the a desired cell density in the cell culture medium, opening the removable lid of the cell culture container, pipetting the a desired volume of cell suspension into the cell culture container, and closing the removable lid of the cell culture container ~~using the lid so as to close the cell culture container.~~

10. (Amended) Use The method according to ~~one or more of Claims claim 1 to 9, characterized in the fact that~~ wherein the cell culture medium comprises RPMI 1640 is used as the cell culture medium.

11. (Amended) Use The method according to ~~one or more of Claims claim 1 to 10, characterized in that~~ wherein the cell culture space comprises at least  $1 \cdot 10^5$  cells per ml of cell culture space are used.

12. (Amended) Use The method according to ~~one or more of Claims claim 1 to 11, characterized in that~~ wherein each cell is at an average distance of 0  $\mu\text{m}$  to 600  $\mu\text{m}$  from the closest membrane in the first and second membrane systems.

13. (Amended) Use The method according to ~~one or more of Claims claim 1 to 12, characterized in that~~ wherein a fluid nutrient medium comprises RPMI 1640 is used.

14. (Amended) Use The method according to ~~one or more of Claims claim 1 to 13, characterized in that~~ wherein the gaseous medium has comprises a  $\text{pO}_2$  of 0 to 160 mmHg and a  $\text{pCO}_2$  of 0 to 115 mmHg.

15. (Amended) Use The method according to ~~one or more of Claims claim 1 to 14, characterized in that~~ wherein the cell culture medium contains comprises a bicarbonate

buffer and the pCO<sub>2</sub> in the gaseous medium added is adjusted so that the pH value of the cell culture medium is between 6.8 and 7.8.

16. (Amended) Use The method according to ~~one or more of Claims claim~~ 1 to 15, characterized in that wherein ~~gaseous metabolic products are removed from the cell culture space by means of the second membrane system~~ removes gaseous metabolic products from the cell culture space.

17. (Amended) Use The method according to ~~one or more of Claims claim~~ 1 to 16, characterized in that wherein the metering of the at least one individual active substances and/or combinations of several active substances are added substance comprises adding the at least one active substance on a time-staggered basis.

18. (Amended) Use The method according to ~~one or several of Claims claim~~ 1 to 17, characterized in that wherein the metering of the at least one active substance dosage is added comprises adding a dose of the at least one active substance to the cell culture ~~chamber~~ space directly or ~~by means of through~~ the first membrane system.

19. (Amended) Use The method according to ~~one or more of Claims claim~~ 1 to 18, characterized in that ~~specification of wherein~~ the active substance concentration-time curve takes place with the is determined based on permeabilities of the first membrane system, ~~by the duration of the active substance administration, and by the active substance concentration.~~

20. (Amended) Use The method according to ~~one or more of Claims claim~~ 1 to 19, characterized in that wherein the cell culture container is kept at 37°C.

21. (Amended) Use The method according to ~~one or more of Claims claim~~ 1 to 18, characterized in that wherein monitoring of the cell vitality is monitored by means of comprises measuring the presence of fluorescent dye converted from a cell vitality dye.

22. (Amended) Use The method according to ~~Claim claim~~ claim 21, ~~characterized in that wherein the cell vitality dye comprises Alamar Blue serves as a cell vitality dye~~

23. (Amended) Use The method according to ~~one or more of Claims claim~~ claim 1 to ~~22, characterized in the fact wherein the monitoring of cell vitality is monitored using~~ comprises at least one sensor.

24. (Amended) Use The method according to ~~Claim claim~~ claim 23, ~~characterized in that wherein the sensor comprises a fluorescence sensor is used.~~

25. (Amended) ~~Device~~ A device for in-vitro testing of active substances in cells, comprising a cell culture container (1) suitable for collecting a cell culture in a cell culture medium with an ~~internal~~ interior chamber (2), ~~with wherein a first means for supplying supply device for introducing at least one nutrient medium and a second supply device for adding at least one gaseous medium are located in the interior space chamber, with the means each having wherein each supply device has a supply side and a removal side, and with a cell culture space being formed between said means supply devices and the an inside wall of the interior chamber, and with the first means supply device in a fluid connection with the supply side connected by to nutrient medium dispensing unit (3) with including at least one nutrient medium container (4), and the second means supply device connected in a fluid connection with the supply side connected by to a gas metering unit (5) with including at least one gas supply container (6), characterized in that wherein the cell culture chamber space has a volume of at most 5 ml and at least 0.1 ml, and that further wherein the device also contains means (7), (8), (9a), (9b), and (9e) comprises an active substance supply container, an active substance dispensing unit, and a line system connecting the active substance supply container with the interior chamber for supplying at least one active substance to the cell culture chamber space, and means for creating an active substance wherein the active substance~~

dispensing unit dispenses the active substance into the cell culture space according to an adjusted active substance concentration-time curve in the cell culture chamber.

26. (Amended) ~~Device~~ The device according to ~~Claim~~ claim 25, characterized ~~in that wherein~~ the first means ~~is in~~ supply device includes a fluid connection on the removal side with a waste container (10).

27. (Amended) ~~Device~~ The device according to ~~Claim~~ claim 25, characterized ~~in that wherein~~ the first means ~~is in~~ supply device includes a fluid connection on the removal side by a recirculation line (11) ~~with the~~ comprising at least one nutrient medium container (4).

28. (Amended) ~~Device~~ The device according to ~~one or more of Claims~~ claim 25 to 27, characterized ~~in that wherein~~ the first means ~~consists of~~ supply device comprises at least one ~~fluid medium suitable for administration~~ membrane suitable for supplying nutrient media.

29. (Amended) ~~Device~~ The device according to ~~one or more of Claims~~ claim 25, to 28 characterized ~~in that wherein~~ the second means supply device consists of comprises at least one membrane suitable for gas exchange.

30. (Amended) ~~Device~~ The device according to ~~one or more of Claims~~ claim 25 to 29, characterized ~~in that wherein~~ cell culture container (1) ~~has~~ comprises a bottom and a lid ~~which bound~~ binding the interior chamber, ~~being~~ are opposite one another, and ~~consist of~~ each comprising a transparent material.

31. (Amended) ~~Device~~ The device according to ~~Claim~~ claim 30, characterized ~~in that wherein~~ the bottom of the cell culture container includes a heating system ~~is integrated into the bottom of cell culture container~~ (1).



32. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25, ~~to 31 characterized in that the~~ wherein the first supply device comprises at least one membrane ~~of the first means that~~ that is a semipermeable membrane or a hydrophilic microporous membrane.

33. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 ~~to 32, characterized in that~~ wherein the second supply device comprises ~~the~~ at least one membrane ~~of the second means that~~ that is an oxygenation membrane.

34. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 ~~to 33, characterized in that~~ wherein the first and second supply devices comprise ~~the~~ membranes ~~of the first and second means that~~ that are hollow fibers.

35. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 ~~to 34, characterized in that~~ wherein the hollow fibers are stacked in several layers in the interior chamber.

36. (Amended) ~~Device~~ The device according to ~~Claim claim~~ 35, ~~characterized in that~~ wherein the maximum distance between the hollow fibers forming each ~~means~~ supply device is between 50 ~~in~~ μm and 600 ~~in~~ μm.

37. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 ~~to 36, characterized in that~~ wherein the cell culture chamber ~~has~~ space comprises a volume of 0.3 ml to 3.0 ml.

38. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 ~~to 37, characterized in that~~ wherein the ~~means~~ supply device for adding the active substance ~~consist of~~ comprises at least one active substance supply container (7), at least one active substance metering device (8), and a system of lines (9) which connects the at least one active substance supply container (7) through ~~an~~ the at least one active substance metering

~~unit (8)~~ device directly (9a) or through ~~first means (9b)~~ the first supply device with the ~~cell culture chamber~~ cell culture space of the cell culture container (1).

39. (Amended) ~~Device~~ The device according to ~~one or more of Claims claim~~ 25 to 38, characterized in that wherein the device ~~has a means for monitoring~~ further includes monitoring for cell vitality.

40. (Amended) ~~Device~~ The device according to ~~Claim claim~~ 39, characterized in that wherein the ~~means for monitoring~~ monitoring of cell vitality consists of comprises at least one sensor.

41. (Amended) ~~Device~~ The device according to ~~Claim claim~~ 40, characterized in that wherein the sensor is comprises a fluorescence sensor.

42. (Amended) ~~Modular~~ A modular active substance testing system comprising at least two devices according to ~~Claims claim~~ 25 to 41.

43. (Amended) ~~Modular~~ The modular active substance testing system according to ~~Claim claim~~ 42, ~~consisting of comprising~~ 6, 24, or 96 devices according to ~~Claims 25 to 41.~~

44. (Amended) ~~Use of the device according to one or more of Claims 25 to 41 or of the modular active substance testing system according to one of Claims 42 or 43 for in vitro testing of the effects of active substances on cells~~ A process for in-vitro testing of the effects of active substances on cells comprising the device according to claim 25.

45. (Amended) ~~Use of the device or of the modular system according to Claim 44, characterized in that~~ The process according to claim 44, wherein the process comprises determining the influence of pharmacokinetics on cell vitality is determined.